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ORIGINAL RESEARCH

Evaluating the Mechanism Underlying Multi-Compound Synergy of Banxia Decoction in the Treatment of Hashimoto's Thyroiditis Based on Network Pharmacology and Molecular Docking

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Objective: We aimed to utilize network pharmacological analysis and molecular docking to elucidate the potential mechanisms of Banxia Decoction (BD) action in the treatment of Hashimoto's thyroiditis (HT).

Materials and Methods: Active compounds and HT-related targets were predicted using databases and the intersection of the results was taken. STRING and DAVID 6.8 tools were used to obtain the protein-protein interaction (PPI) network and perform GO and KEGG evaluations, respectively. Discovery Studio 2017 R2 was utilized to perform molecular docking and RT-qPCR was conducted to confirm hub gene expressions in clinical samples.

Results: A total of 136 active compounds in BD were screened, and 74 potential targets related to HT were identified in BD. Further, 17 key targets in the PPI network were identified and HIF1A, EP300, PRKCA, and TERT were included for subnet analysis. Next, a network of "Chinese medicine-active compound-potential target-signal pathway" was obtained and the HIF-1 signaling pathway was identified as the key pathway. Finally, 8 active compounds and their stable binding to target proteins were confirmed by molecular docking; MAPK3, SRC, TERT, and HIF1A were upregulated in HT relative to the goiter samples.

Conclusion: The integration of network pharmacology and molecular docking provides a systematic framework for exploring the multi-component and multi-target characteristics of BD in HT, underscores the therapeutic potential of BD in HT by targeting genes and pathways involved in immune regulation and oxidative stress. These findings not only enhance our understanding of BD's pharmacological mechanisms but also lay the groundwork for the development of novel therapeutic strategies for HT.

Keywords: Hashimoto's thyroiditis, Banxia decoction, network pharmacology, molecular docking

Introduction

Hashimoto's thyroiditis (HT), or chronic lymphocytic thyroiditis, is an autoimmune disease of the thyroid characterized by diffuse enlargement, lymphocyte infiltration, and elevation in anti-thyroid peroxidase antibody and thyroglobulin antibody levels, commonly causing hypothyroidism in adults.[1] In recent years, owing to an increase in societal pressure, implementation of salt iodization in China, and an increase in environmental pollution and ionizing radiation, the incidence of HT has increased rapidly, with an incidence rate of 0.3–1.5 cases per 1000 people.[2,3] Apart from neck compression, systemic symptoms caused by hypothyroidism, and non-specific rheumatic manifestations related to autoimmune diseases, the relationship between HT and thyroid cancer deserves more attention from clinicians for effective treatment.[4] Although the imbalance between cellular and humoral immunity is crucial to its pathogenesis, the specific mechanism for HT remains unclear, resulting in a major obstacle to developing its fundamental etiology and

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treatment. At present, the standard treatment for HT involves levothyroxine replacement therapy, aimed at correcting hypothyroidism. However, this approach does not address the root causes of HT, such as autoimmune dysfunction and inflammation. Thus, the need for effective therapies targeting the underlying immune dysfunction is critical. Traditional Chinese medicine (TCM), recognized by the World Health Organization as a valuable component of complementary and alternative medicine, provides an important alternative treatment strategy.[5] TCM uses multi-ingredient formulations that act on multiple targets, which often results in enhanced therapeutic effects and reduced side effects.[6] For a long time, Chinese medicine has been crucial in treating HT, as it reduces autoimmune antibodies, regulates immune responses, maintains thyroid function, and relieves clinical symptoms.[7,8] Despite this, the complexity of TCM formulations and the challenge of identifying their precise mechanisms have hindered a thorough understanding of their efficacy.

In the context of HT, Banxia Decoction (BD), a classical TCM prescription recorded in the "Taiping Shenghui Fang" from the Song Dynasty in China, has been widely used for thyroid-related disorders. BD is traditionally known for its ability to regulate Qi, resolve phlegm, and dispel congestion, aligning with the Western understanding of HT as an autoimmune disease. A review of clinical studies has shown that Pinellia ternata (the main component of BD) is one of the most frequently used herbs in the treatment of thyroid diseases, accounting for approximately 75% of clinical cases.[9] This indicates the significant role of Pinellia ternata in treating HT. Despite variations in BD formulations, especially in terms of compounds, the theoretical foundation remains similar, and the prescription with Pinellia ternata as the main component is widely reported in Chinese medical literature.

While the effects of Pinellia ternata on tumors, especially thyroid cancer, have been studied, its role in treating HT, particularly in the context of BD, remains underexplored. At our hospital, traditional Chinese medicine formulas, including BD, have been used as adjunctive treatments for HT. Given that HT is an immune-related disease, studying its pathogenesis through traditional cell-based models is challenging. Network pharmacology offers a promising approach to investigate the mechanisms of TCM compounds in treating HT. This study aims to explore these mechanisms and lay the foundation for future animal experiments to validate the findings and deepen our understanding of HT's pathogenesis.[10]

Materials and Methods

Collection and Preliminary Screening of Active Compounds in BD

The main compounds in BD, including 12 Chinese medicines, namely Pinellia ternata, Belamcanda chinensis, Arctium lappa, Almond, Tylophora asthmatica, Akebia quinata, Platycodon grandiflorus, Khumbu, Citrus aurantium, Licorice, Areca, and Tuckahoe were extracted utilizing Traditional Chinese Medicine Systems Pharmacology (TCMSP, https://old.tcmsp-e.com/index.php)[11] Database and Analysis Platform, and Traditional Chinese Medicine Integrated Database (TCMID, http://www.megabionet.org/tcmid/).[12] While the main compounds in Tylophora asthmatica were obtained from traditional Chinese medicine literature, PubChem (https://pubchem.ncbi.nlm.nih.gov/)[13] was used to search and standardize the structures and supplement the PubChem ID. ADMET refers to the absorption, distribution, metabolism, excretion, and toxicity of drugs, and is key to pharmacokinetic research for designing and screening drugs.[14] The ADMET Descriptors module in Discovery Studio 2017R2 was used to predict the ADMET parameters of Chinese medicine compounds. The active compounds in BD were preliminarily screened according to the human intestinal absorption (ADMET absorption level) and ADMET aqueous solubility; ADMET absorption levels were set at 0, 1, and 2, while ADMET aqueous solubility at 1, 2, 3, and 4. The workflow was illustrated in Figure 1.

Prediction and Screening of Targets in Active Compounds

The aforementioned standardized active compound structures were imported into Drugbank (https://go.drugbank.com/),[15] Therapeutic Target Database (http://db.idrblab.net/ttd/)[16] and SwissTargetPrediction (http://www.swisstargetprediction.ch/)[17] were used to predict the related targets and obtain the active compound target database of BD.

Based on the methodology of Fu et al,[18] targets were further predicted. Briefly, deep learning and Bayesian network algorithm were adopted to test and score the active compounds of BD and their targets in the constructed database. The network topological parameters were computed based on the scores to screen targets of the active compounds.

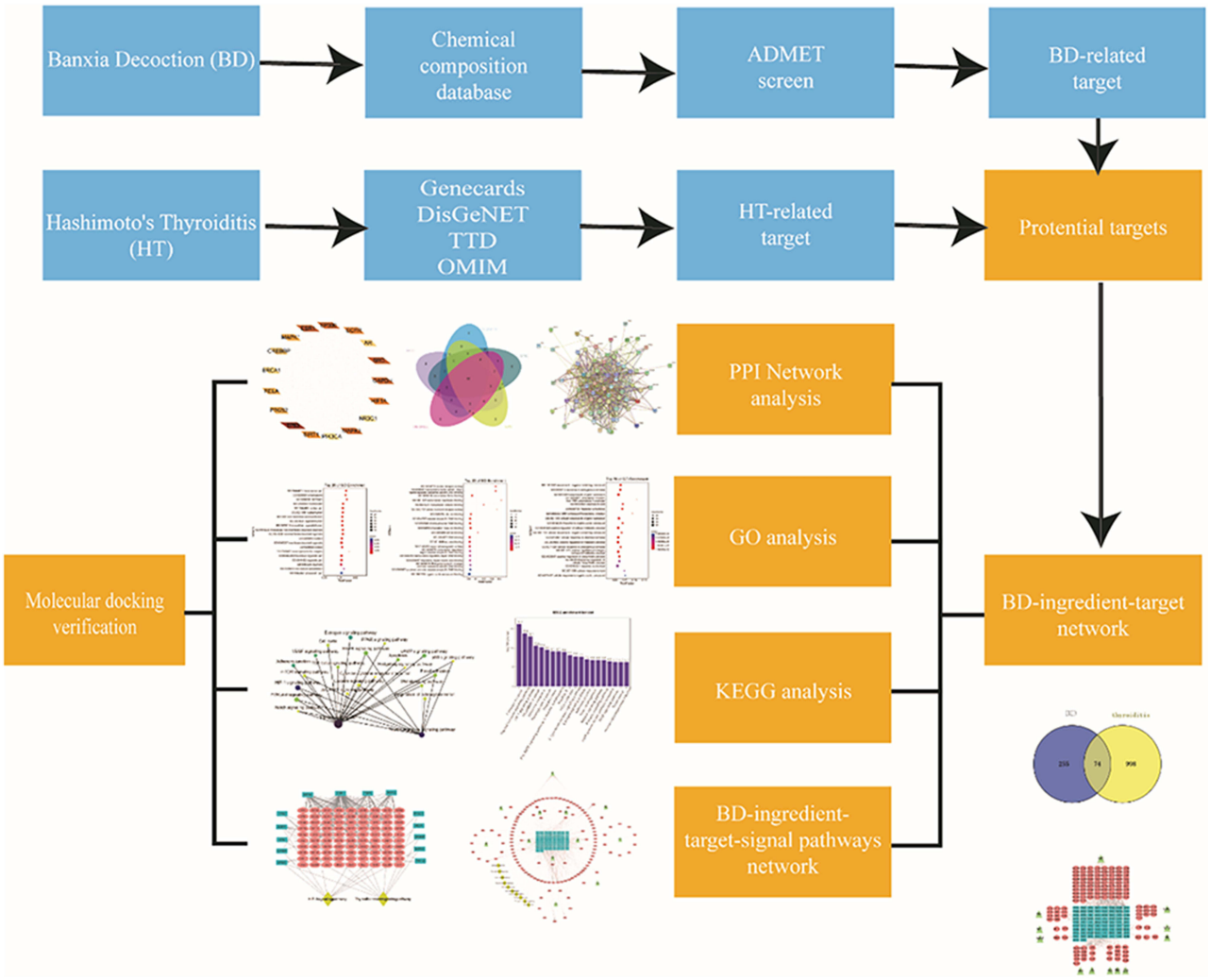

Figure 1 Workflow of the study.

Retrieval of HT Targets

PubMed was queried using "Hashimoto's thyroiditis" as the MeSH term and all relevant terms were collected and searched on DisGeNet (https://www.disgenet.org/), GeneCards (https://www.genecards.org/), Therapeutic Target databases (http://db.idrblab.net/ttd/), and OMIM (https://omim.org/) for obtaining related targets.[16,19–21] Finally, the targets obtained from the 4 databases were subjected to intersection; duplicates or false-positive genes were discarded, and a disease target database was established for HT.

Compound-Disease Target Network Construction and Hub Gene Screening

After taking the intersection of the active compound targets and disease targets, the common targets were imported to the STRING database (https://cn.string-db.org/).[22] The species was set as "Homo sapiens" and the confidence level at ≥0.400 to construct a protein-protein interaction (PPI) network of the intersection (common) targets. The obtained PPI network was imported into Cytoscape for visualization.[23] Using plug-ins, including maximum neighborhood component (MNC), degree correlation (DEGREE), edge percolated component (EPC), closeness centrality (CLONESS), and maximal clique centrality (MCC) in the cytoHubba[24], the hub genes were detected; MCODE[25] was used to obtain gene clusters according to the relationship between the proteins in the network and derive sub-networks. Finally, differentially expressed genes in each gene cluster were extracted and the key biological processes were assessed in the sub-networks.

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GO and KEGG Pathway Analyses and Construction of "Active Compound-Potential Target-Signal Pathway" Network

The candidate hub genes were imported into DAVID 6.8 tool (https://david.ncifcrf.gov/)[26] for gene ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, following which the biological processes and signaling pathways relevant to the hub genes were screened. P<0.05 indicated a statistically significant difference. Cytoscape was used to construct and visualize the "active compound-potential target-signal pathway" network.

Molecular Docking and Histological Confirmation

The 3-dimensional (3D) structures of the main active compounds were imported into PubChem according to the PubChem ID and high-resolution crystal structures of the HT targets were obtained from the Protein Data Bank (PDB, http://www.rcsb.org/pdb/).[27] Further, these targets were imported into Discovery Studio 2017 R2,[28] and CDOCKER in the Receptor-Ligand International module was used to accurately dock and analyze HT targets and active compounds. Random Conformations were set at 10, Pose Cluster Radius at 0.5, Orientations to Refine at 10, and default values were adopted for other parameters.[29] This study complies with the Declaration of Helsinki and was approved by the Zhuzhou Central Hospital of Ethics Committee (Approval No.: [KY2025011-01]).

From December 2020 to December 2021, 59 clinical samples were collected, including 21 cases of HT: 4 males and 17 females, aged 21~57 (41.6±12.0) years, and 38 cases of goiter or normal thyroid: 6 males and 32 females, aged 17~67 (44.6±12.1) years. All patients underwent thyroid lobectomy or total thyroidectomy owing to an overly enlarged thyroid or suspected thyroid nodules. Patients with postoperative pathological diagnosis of HT, nodular goiter, or normal thyroid were included; malignant cases were excluded. All data were de-identified.

Total RNA was extracted using TriZol (Sigma-Aldrich Co., Ltd., Shanghai, China), following which reverse transcription was performed using the SureScript cDNA synthesis kit (GeneCopoeia Co., Ltd., Guangzhou, China). A two-step real-time quantitative PCR was performed using the BlazeTaq qPCR kit (GeneCopoeia Co., Ltd., Guangzhou, China) on the LightCycler 96 PCR system (Roche Co., Ltd., Rotkreuz, Switzerland). ΔCT was calculated using the ACTB-adjusted Ct values (the number of cycles required for the fluorescence intensity to reach a given threshold) and $2^{-\Delta\Delta CT}$ was used to quantify the mean target gene expression for all samples in each group. Primers used in PCR can be find in Table 1.

Statistical Analysis

Statistical analysis was performed and violin plots were drawn using R (version 4.1.1, https://www.r-project.org/). Gene expression was presented as the mean ± SD of three independent experiments and plotted on a violin diagram. The *t*-test was used for comparison between groups for normally distributed data and homogeneity of variance. A corrected *t*-test was used when the normal distribution was not homogeneous for variance; the Mann–Whitney *U*-test was used for non-normal distributions. Statistical significance was determined by a two-tailed P-value <0.05.

Results

Active Compounds in BD

After searching the database, 186 active compounds in BD were obtained, comprising phenylpropanoids, flavonoids, alkaloids, and terpenes. These compounds were predicted based on ADMET parameters, and 139 active compounds were screened, including 4 in Pinellia, 13 in Shegan, 2 in Burdock, 11 in Almonds, 2 in Antelope horns, 4 in Mutong, 3 in Areca, 3 in Platycodons, 3 in Khumbu, 4 in Civic aurantium, 79 in Licorice, and 11 in Tuckahoe (Supplementary Table 1).

Screening of Targets Related to HT in BD

A total of 329 active compound targets of BD were identified based on the target database. Among HT-related targets, 1037 were obtained from GeneCards, 2 from OMIM, 104 from DisGeNET, and 10 from the Therapeutic Target Database. After merging and removing duplicates, 1072 targets were obtained. After conducting the intersection of 329 active compound targets of BD with 1072 targets of HT, 74 potential targets related to HT in BD (Figure 2A) were identified and used to construct the "Traditional Chinese Medicine-active compound-target interaction" network (Figure 2B).

Table 1 Primers Used in PCR

Symbol	Sequence
TP53-F	5'-GATGGTGGTACAGTCAGAGCC-3'
TP53-R	5'-CCTCAGCATCTTATCCGAGTGG-3'
ESR1-F	5'-AACAGCCTCCTGTCTACCGA-3'
ESR1-R	5'-CATGGACCTTTCGAGCCTGT-3'
SRC-F	5'-GGACAGACAGGCTACATCCC-3'
SRC-R	5'-TCTGACTCCCGTCTGGTGAT-3'
MAPK3-F	5'-CAGACTCCAAAGCCCTTGACC-3'
MAPK3-R	5'-CCTTCAGCCGCTCCTTAGGTA-3'
GADPH-F	5'-TCGGAGTCAACGGATTTGGT-3'
GADPH-R	5'-TTCCCGTTCTCAGCCTTGAC-3'
EGFR-F	5'-AGGCACGAGTAACAAGCTCAC-3'
EGFR-R	5'-ATGAGGACATAACCAGCCACC-3'
PRKCA-F	5'-GTCCACAAGAGGTGCCATGAA-3'
PRKCA-R	5'-AAGGTGGGGCTTCCGTAAGT-3'
HIF1A-F	5'-GAACGTCGAAAAGAAAAGTCTCG-3'
HIF1A-R	5'-CCTTATCAAGATGCGAACTCACA-3'
EP300-F	5'-CCAAACCAGATGATGCCTCG −3'
EP300-R	5'-TTTGCCGGGGTACAATAGGG-3'
TERT-F	5'-AGCCACGTCTCTACCTTGAC-3'
TERT-R	5'-CTCATTCAGGGAGGAGCTCT-3'
ACTB-F	5'-CATGTACGTTGCTATCCAGGC-3'
ACTB-R	5'-CTCCTTAATGTCACGCACGAT-3'

PPI Network and Hub Genes

There were 74 nodes (target proteins) and 732 edges (protein interactions) in the PPI network imported into Cytoscape (Figure 3A). The top 20 results based on the five parameters (DEGREE, MNC, CLONESS, EPC, and MCC) were analyzed. After the intersection, 17 key targets were screened (Figure 3B; Tables 2 and 3). Topology algorithms were utilized to calculate and analyze the network structure and weigh the connection between nodes (Figure 3C); the topological parameters of 17 key targets are shown in Table 2. Subnet 1 was centered on HIF1A and EP300, and

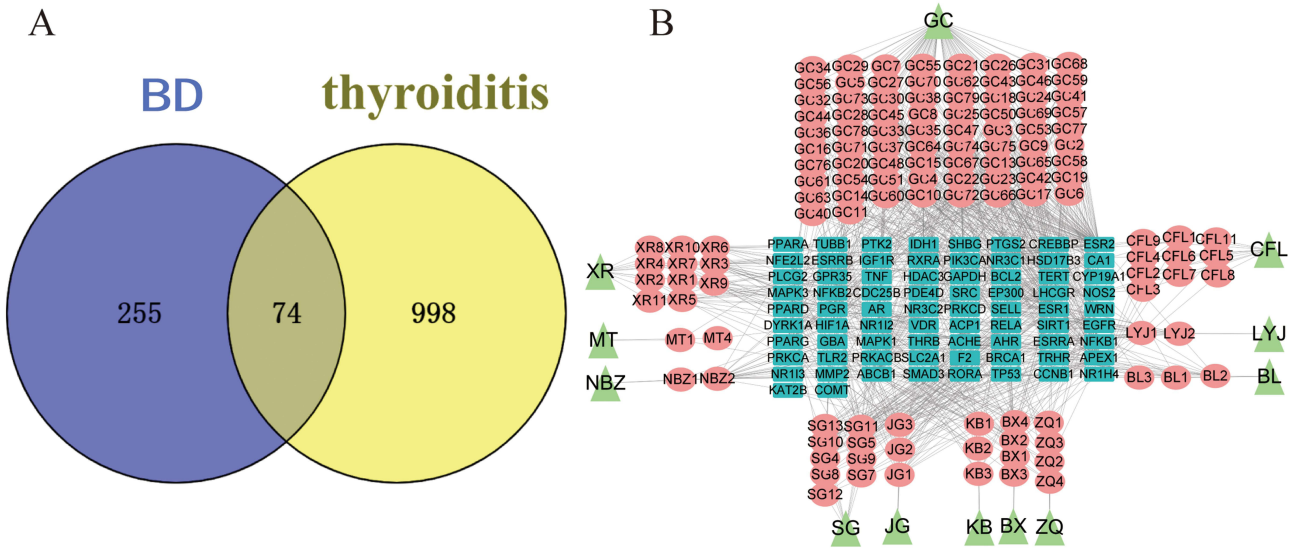

Figure 2 Screening of targets related to HT in BD. (**A**), Intersection of BD active compound targets and HT targets. (**B**), Traditional Chinese medicine-active compound-target interaction network.

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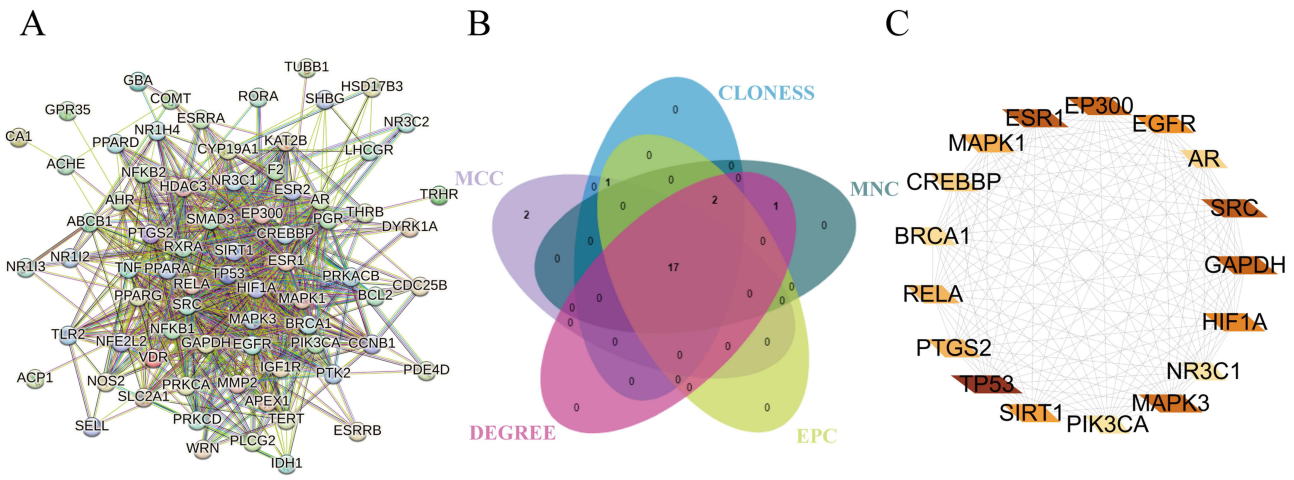

Figure 3 PPI network. (**A**), All target protein interaction network. (**B**), MNC, DEGREE, MCC, CLONESS, EPC five algorithms Top 20 target Venn diagram. (**C**), key target protein interaction network diagram.

important targets connected to them included PIK3CA, SRC, and AR (Figure 4A); the core of subnet 2 comprised PRKCA and TERT, and points included SLC2A1, NFE2L2, PTGS2 are important targets connected to them (Figure 4B).

GO Annotation, KEGG Pathway Analysis, and "Active Compound-Potential Target-Signal Pathway" Network

GO annotation yielded 284 biological processes, 26 cellular components, and 85 molecular functions (Figure 5A-C, Table 4). According to the P-value and the number of enriched targets, the top 20 GO terms were selected and further evaluated. Among biological processes, the key targets were enriched in positive regulation of the cellular metabolic process, response to organic substance, response to oxygen-containing compound, and response to endogenous stimulus;

Table 2 Cytohubba Algorithm Calculation Results Sorting

Rank	MCC	CLONESS	MNC	EPC	DEGREE
1	TP53	TP53	TP53	TP53	TP53
2	CCNB1	EGFR	EGFR	EGFR	EGFR
3	EGFR	CREBBP	CREBBP	CREBBP	CREBBP
4	CREBBP	AR	AR	AR	AR
5	AR	PTGS2	PTGS2	PTGS2	PTGS2
6	PTGS2	PIK3CA	PIK3CA	PIK3CA	PIK3CA
7	PGR	SIRT1	IGF1R	SIRT1	IGF1R
8	PIK3CA	SRC	SIRT1	SRC	SIRT1
9	SIRT1	SMAD3	SRC	SMAD3	SRC
10	SRC	MAPK1	MAPK1	MAPK1	MAPK1
11	SMAD3	RELA	RELA	RELA	RELA
12	MAPK1	TNF	TNF	TNF	TNF
13	RELA	ESR1	ESR1	ESR1	ESR1
14	ESR1	PPARG	PPARG	PPARG	PPARG
15	BRCA1	BRCA1	BRCA1	BRCA1	BRCA1
16	HIF1A	HIF1A	HIF1A	HIF1A	HIF1A
17	GAPDH	GAPDH	GAPDH	GAPDH	GAPDH
18	NR3C1	NR3C1	NR3C1	NR3C1	NR3C1
19	MAPK3	MAPK3	MAPK3	MAPK3	MAPK3
20	EP300	EP300	EP300	EP300	EP300

Table 3 Topological Parameter Analysis of Key Targets

Name	Closeness	Betweenness	Degree
TP53	0.72028	0.089257	65
MAPK3	0.691275	0.075607	58
EGFR	0.664516	0.053446	54
SRC	0.668831	0.062957	54
MAPK1	0.668831	0.051797	53
TNF	0.664516	0.071755	52
HSP90AA1	0.647799	0.042616	49
PTGS2	0.651899	0.065981	49
CXCL8	0.647799	0.050733	48
ESR1	0.64375	0.043074	47
CCND1	0.631902	0.026968	47
EP300	0.605882	0.015898	41
RELA	0.602339	0.010473	39
BRCA1	0.575419	0.016872	36
PIK3CA	0.588571	0.019866	35
AR	0.581921	0.02361	34
HIF1A	0.578652	0.005801	33

molecular functions in sequence-specific DNA binding, transcription factor binding, transition metal ion binding, and zinc ion binding; cellular components in organelle lumen and nucleus.

The top 10 enriched pathways included thyroid hormone, HIF-1, estrogen, adherens junction, prolactin, VEGF, FoxO, ErbB, sphingolipid, and neurotrophin signaling (Figure 6A–B, Table 5). Moreover, based on these signaling pathways, together with the aforementioned Chinese medicines, active compounds, and potential targets, we constructed a network of "Chinese medicine-active compound-potential target-signal pathway" (Figure 7A), whereby Citrus aurantium, Pinellia, Areca, Khumbu, and Platycodons exerted the most important effects. According to MCC-based computation results (cytohubba), the HIF-1 signaling pathway was the key in this network (Figure 7B).

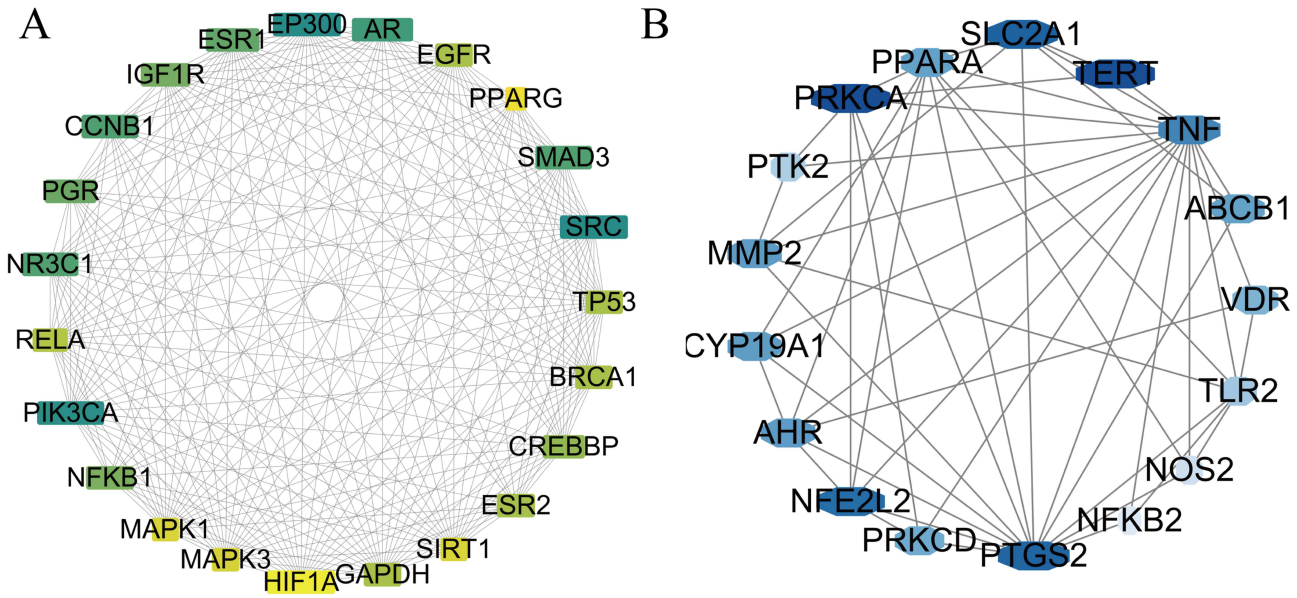

Figure 4 The MCODE subnet analysis. (**A**), Subnet 1. (**B**), Subnet 2.

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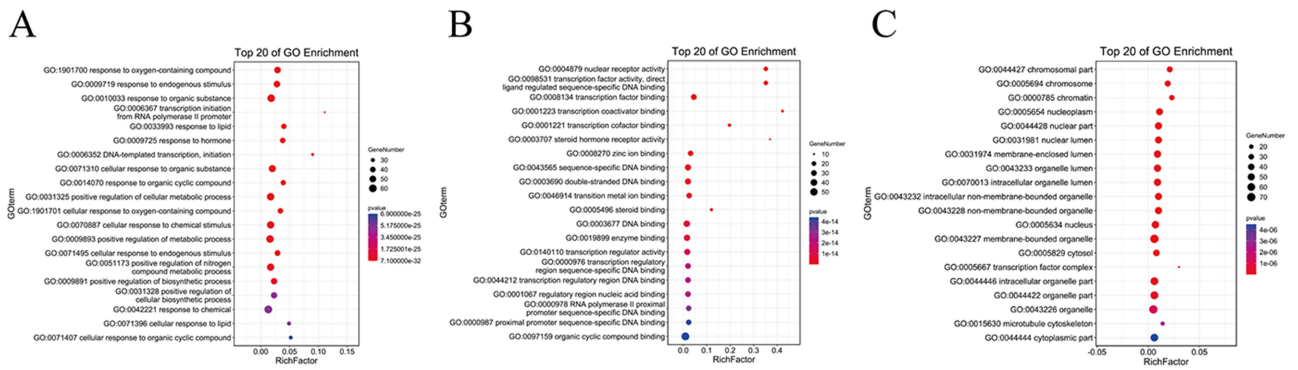

Figure 5 GO enrichment of key targets. (**A**), BP analysis result. (**B**), MF analysis result. (**C**), CC analysis result.

Molecular Docking and Histological Confirmation

Docking between the hub genes and the related compounds showed that the CDOCKER energies across the 8 active compounds and the target proteins were negative, indicating stable binding (Table 6). Among them, EGFR and kaempferol

Table 4 GO Terms

Class	GO	Term	Count	P-value
Biological Process	GO:1901700	Response to oxygen-containing compound	48	7.06E-32
	GO:0009719	Response to endogenous stimulus	48	1.16E-31
	GO:0010033	Response to organic substance	59	1.39E-30
	GO:0006367	Transcription initiation from RNA polymerase II promoter	25	1.49E-29
	GO:0033993	Response to lipid	38	3.16E-29
	GO:0009725	Response to hormone	39	3.16E-29
	GO:0006352	DNA-templated transcription, initiation	26	2.05E-28
	GO:0071310	Cellular response to organic substance	53	5.25E-28
	GO:0014070	Response to organic cyclic compound	37	5.92E-28
	GO:0031325	Positive regulation of cellular metabolic process	57	1.72E-27
Molecular Function	GO:0004879	Nuclear receptor activity	19	4.03E-33
	GO:0098531	Transcription factor activity, direct ligand regulated sequence-specific DNA binding	19	4.03E-33
	GO:0008134	Transcription factor binding	30	3.57E-24
	GO:0001223	Transcription coactivator binding	11	1.50E-20
	GO:0001221	Transcription cofactor binding	13	4.08E-19
	GO:0003707	Steroid hormone receptor activity	10	4.68E-18
	GO:0008270	Zinc ion binding	26	6.35E-17
	GO:0043565	Sequence-specific DNA binding	34	9.04E-17
	GO:0003690	Double-stranded DNA binding	33	6.70E-16
	GO:0046914	Transition metal ion binding	28	7.16E-16
Cellular Component	GO:0044427	Chromosomal part	34	3.63E-17
	GO:0005694	Chromosome	35	9.02E-17
	GO:0000785	Chromatin	28	7.32E-15
	GO:0005654	Nucleoplasm	47	2.87E-14
	GO:0044428	Nuclear part	50	1.03E-13
	GO:0031981	Nuclear lumen	47	7.33E-13
	GO:0031974	Membrane-enclosed lumen	52	1.37E-12
	GO:0043233	Organelle lumen	52	1.37E-12
	GO:0070013	Intracellular organelle lumen	52	1.37E-12
	GO:0043232	Intracellular non-membrane-bounded organelle	49	3.58E-12

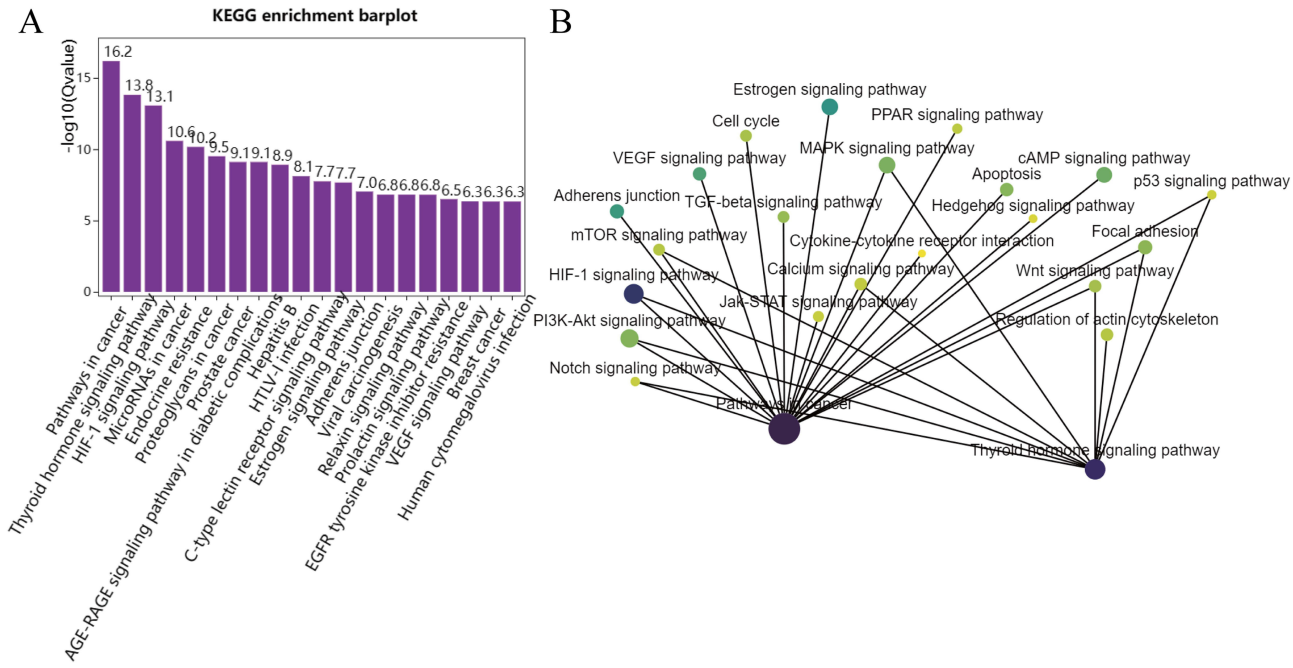

Figure 6 KEGG pathway enrichment (**A**), KEGG pathway annotation classification. (**B**), KEGG pathway network diagram.

showed the lowest CDOCKER energy of −137.87; ESR1 showed a good binding ability to 7 active compounds, and TP53, SRC, GAPDH, MAPK3, PRKCA, and TERT combined stably with baicalein, quercetin, licochalcone B, quercetin, diisooctyl succinate, and kaempferol, respectively. These docking modes are shown in Figure 8. The expression levels of the 10 hub genes were confirmed in HT and goiter samples (Figure 9). The expression levels of 4 out of the 10 genes (MAPK3, SRC, TERT, and HIF1A) were remarkably elevated in HT relative to goiter samples.

Discussion

The network pharmacology uses multi-omics and network analysis techniques to integrate information from bioinformatics, systems biology, and multi-pharmacology holistically, thus offering an approach to unveil the complex multiple interactions among drugs, targets, and diseases.[30,31] The integral and systematic traits of network pharmacology are consistent with traditional Chinese medicine, providing a new solution for bridging the theoretical gap between traditional Chinese and Western medicine and developing new drugs.[32,33] The multi-component synergistic effects of BD in the treatment of irritable bowel syndrome have been previously confirmed by network pharmacology.[34]

Table 5 Enrichment of KEGG Pathway

Pathway	Count	*P*-value
Thyroid hormone signaling pathway	17	1.38E-16
HIF-1 signaling pathway	16	1.15E-15
Estrogen signaling pathway	12	1.15E-09
Adherens junction	9	5.39E-09
Prolactin signaling pathway	9	1.11E-08
VEGF signaling pathway	8	3.57E-08
FoxO signaling pathway	10	1.57E-07
ErbB signaling pathway	8	5.04E-07
Sphingolipid signaling pathway	9	6.88E-07
Neurotrophin signaling pathway	9	7.36E-07

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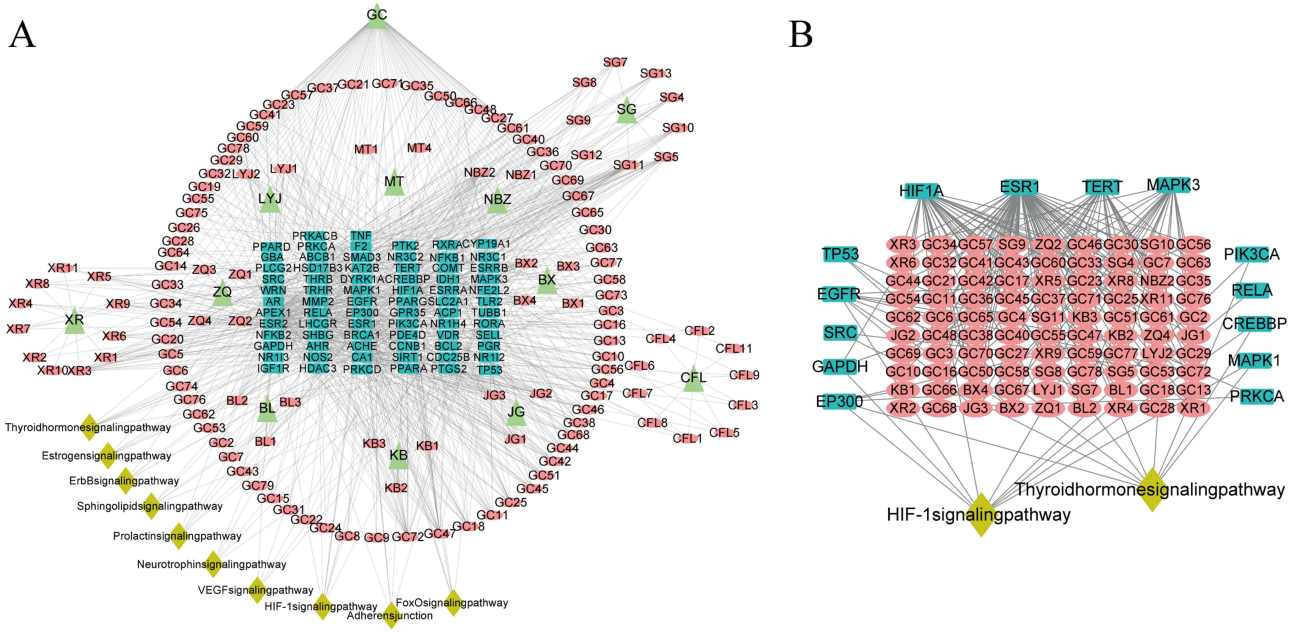

Figure 7 BD-compound-target-pathway diagram (**A**), BD- compound-target-pathway diagram. (**B**), key target-component diagram.

Some preliminary experiments confirm that traditional Chinese medicines exert therapeutic effects against HT. Hou Yi et al report that Jiayan Kangtai granules can protect against autoimmune thyroiditis in a rat model by regulating the balance of Th17/Treg cells.[35] Bing'e Ma et al show that Yanghe decoction suppresses experimental autoimmune thyroiditis in rats by improving NLRP3 inflammasome and immune disorders.[36] The active compounds of Chinese

Table 6 Molecular Docking Between Key Targets and Related Compounds

Target	PDB ID	Compound	CDOCKER Energy
TP53	5HOU	Baicalein	−10.9873
		Dinatin	−5.0247
ESR1	4ZNH	Luteolin	−22.6955
		Kaempferol	−37.1051
		Quercetin	−37.218
		Dinatin	−37.4256
		Glycyrol	19.9689
		Licochalcone B	−37.8944
		(+)-Epitaxifolin	−25.7716
SRC	1Y57	Quercetin	−38.3636
GAPDH	1U8F	Licochalcone B	−34.1484
MAPK3	2ZOQ	Luteolin	−38.6381
		Baicalein	−37.7433
		Quercetin	−57.8526
		Dinatin	−51.8096
		(+)-Epitaxifolin	−38.5834
EGFR	5XWD	Luteolin	−47.7778
		Kaempferol	−137.87
		Baicalein	−52.1556
		Quercetin	−115.496

(*Continued*)

Table 6 (Continued).

Target	PDB ID	Compound	CDOCKER Energy
PRKCA	3IW4	Diisooctyl succinate	−59.1465
TERT	7BG9	Luteolin	−23.0698
		Kaempferol	−64.2461
		Baicalein	−20.044
		Quercetin	−44.2354
		Dinatin	−42.7057
		(+)-Epitaxifolin	−19.0275

medicines, diosgenin, and luteolin, may inhibit HT progression by activating the cAMP/PKA/Creb pathway and inhibiting the IL-6/ STAT1 pathway, respectively.[37,38] Jie Chen et al confirm that ginsenoside exerts a biphasic regulatory effect on IL-4 and regulates the expression of T-bet and GATA-3 to protect against HT in rats.[39] The medical records of thyroid diseases treated by modern Chinese medicine have been collated and analyzed; Pinellia ternata is the most used clinical drug, accounting for approximately 75% of the total cases,[9] indicating its important role in the treatment of HT.

The therapeutic effects of BD in autoimmune diseases such as ulcerative colitis have been investigated.[40,41] Our study provides insights into the therapeutic mechanisms of BD in the treatment of HT through network pharmacology and molecular docking analyses. The identification of hub genes such as TP53, MAPK3, EGFR, SRC, ESR1, HIF1A, and PRKCA, coupled with active compounds like baicalein, quercetin, licochalcone B, diisooctyl succinate, and kaempferol, highlights the multi-component and multi-target nature of BD.

The active compounds exert their effects through various mechanisms after binding to the hub genes. They can directly influence hub gene function by enzymatically inhibiting or activating targets like SRC, MAPK3, and PRKCA, or by regulating transcription factors such as HIF1A, which subsequently alters the expression of downstream genes involved in HT pathogenesis. These hub genes are central to critical pathways such as the HIF-1 signaling pathway, thyroid hormone pathway, and inflammatory pathways, where their modulation can affect hypoxia responses, thyroid function, and autoimmune activity. For example, the regulation of HIF1A can alter hypoxia-induced inflammation,[42] while EGFR and MAPK3 modulation may improve thyroid hormone signaling and immune balance.[43] Furthermore, BD compounds such as quercetin and kaempferol possess strong antioxidant properties, neutralizing reactive oxygen species (ROS) and mitigating oxidative stress-induced thyroid damage.[44] Mechanisms involving TP53 highlight the regulation of apoptosis to remove damaged cells, while modulation of TERT can suppress abnormal thyroid tissue proliferation.[45] Beyond these, the active compounds can restore cellular metabolism by normalizing glycolysis and oxidative phosphorylation, as influenced by GAPDH and MAPK3.[46] Stable binding between these compounds and hub genes, as demonstrated in molecular docking, may also stabilize protein-protein interactions, fine-tuning signaling networks to prevent aberrant immune and inflammatory responses.[47]

To elucidate the mode of action of predicted targets and active compounds at the molecular level, the above-mentioned compounds were docked onto the relevant targets. Some studies based on network pharmacology and molecular docking have reported multi-component synergistic mechanisms of traditional Chinese medicine in the treatment of diseases,[48,49] and significant progress has been made. The lower the binding energy score, the more stable the conformation, and binding energy ≤ -5.0 kcal·mol−1 is considered the criterion for good binding between the ligands and the corresponding receptors.[50] The key compounds obtained by molecular docking were baicalein, licochalcone B, kaempferol, diisooctyl succinate, and quercetin, which are also essential for BD to exert its effects. In previous studies, most of these active compounds have shown therapeutic effects against several autoimmune diseases.[51–53] Our findings link these to HT and provide leading theoretical clues for subsequent experimental studies.

Regarding the integration of BD into clinical practice, this study lays the groundwork for future clinical trials and in vivo studies to validate its efficacy and safety. The multi-target and multi-pathway approach of BD, positions it as a promising candidate for use in conjunction with conventional therapies, such as levothyroxine replacement, to offer a more comprehensive treatment for HT. The findings from this study also provide clinicians with a deeper understanding

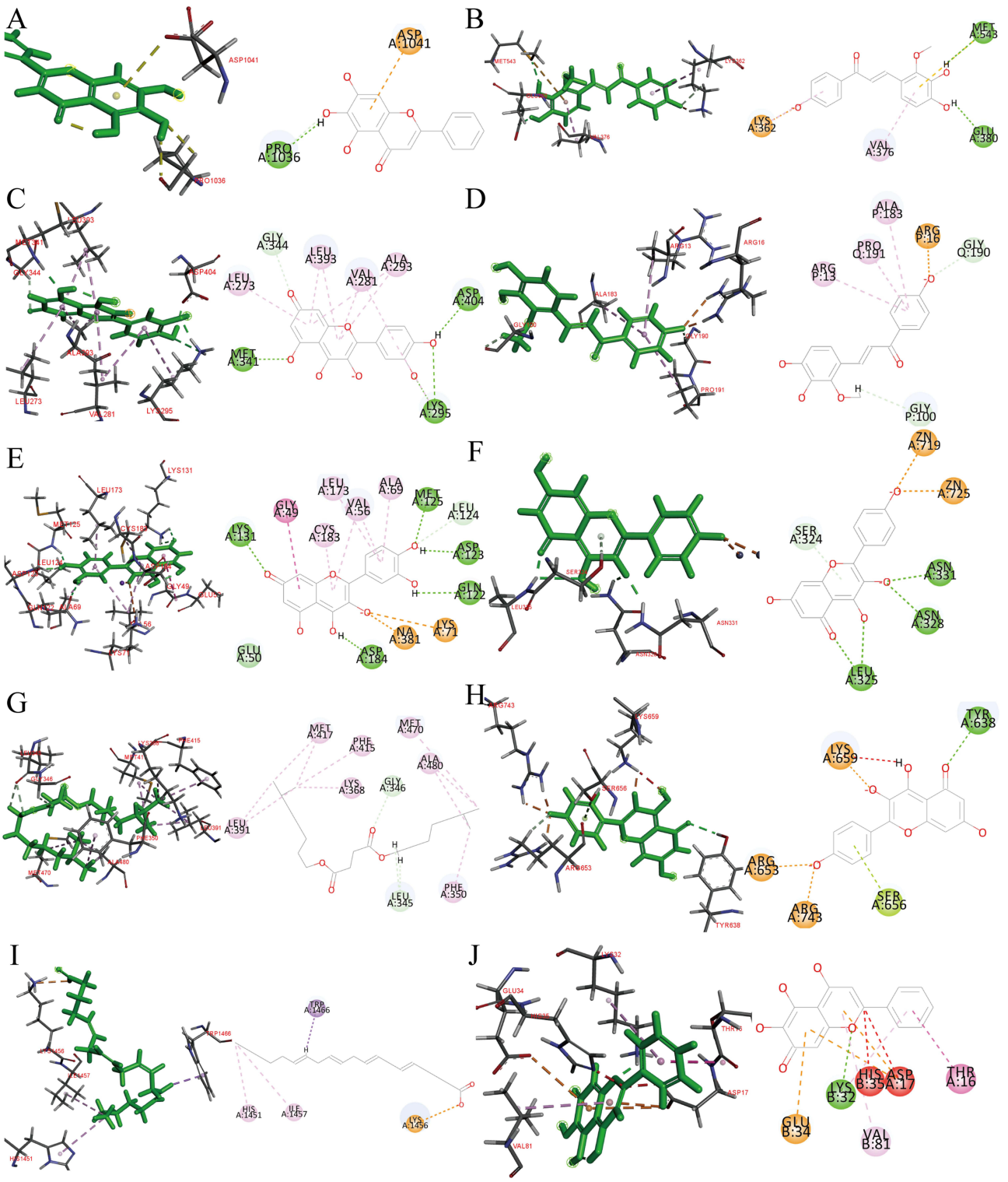

Figure 8 3D and 2D simulation diagram of the molecular docking. (**A**), Docking images of TP53 and Baicalein. (**B**), Docking images of ESR1 and Licochalcone B. (**C**), Docking images of SRC and Quercetin. (**D**), Docking images of GAPDH and Licochalcone B. (**E**), Docking images of MAPK3 and Quercetin. (**F**), Docking images of EGFR and kaempferol. (**G**), Docking images of PRKCA and Diisooctyl succinate. (**H**), Docking images of TERT and kaempferol. (**I**), Docking images of EP300 and arachidonic acid. (**J**), Docking images of HIF1A and Baicalein.

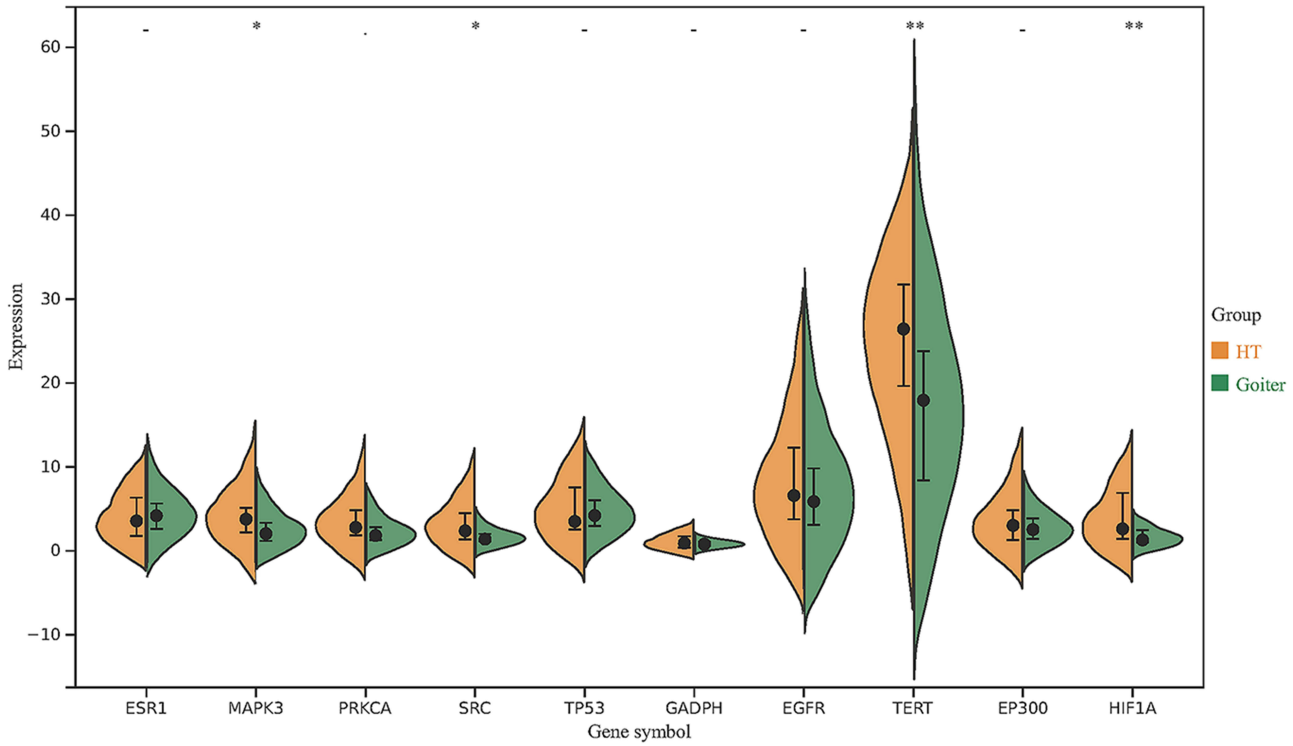

Figure 9 Histological confirmation of key targets. Different color blocks represent the range of gene expressions and data distributions in different groups, and black dots and bars represent median and interquartile range. *p<0.05, **p < 0.01, - p > 0.05.

of the molecular mechanisms behind BD's action, potentially guiding personalized treatment approaches based on individual immune and metabolic profiles.

While these findings are promising, it is important to acknowledge the limitations of the current study. The in silico nature of network pharmacology and molecular docking provides predictive insights but lacks experimental validation. Future research should focus on in vitro and in vivo studies to confirm these mechanisms and assess the clinical relevance of the identified compounds and targets. Furthermore, the complexity of HT pathogenesis necessitates a more comprehensive approach, incorporating advanced techniques such as single-cell transcriptomics and multi-omics analyses.[54]

Conclusions

In conclusion, this study underscores the therapeutic potential of BD in HT by targeting multiple genes and pathways involved in immune regulation and oxidative stress. The integration of network pharmacology and molecular docking provides a systematic framework for exploring the multi-component and multi-target characteristics of traditional Chinese medicine. These findings not only enhance our understanding of BD's pharmacological mechanisms but also lay the groundwork for the development of novel therapeutic strategies for HT.

Data Sharing Statement

All data are available in the manuscript and they are showed in tables, figures.

Ethics Approval and Consent to Participate

This study complies with the Declaration of Helsinki and was approved by the Zhuzhou Central Hospital of Ethics Committee (Approval No.: [KY2025011-01]).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

All authors declare no competing interest.

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